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Ciliated Protozoa in the Rumen of Holstein-Friesian Cattle (*Bos taurus taurus*) in Hokkaido, Japan, with the Description of Two New Species

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ABSTRACT—The composition of rumen ciliates in the Holstein-Friesian cattle bred in Hokkaido, Japan was surveyed. Of 50 species with 19 formae under 15 genera identified, two new species were recognized, then described as *Entodinium okoppensis* and *Ostracodinium munham*. *Entodinium okoppensis* may be classified further into such four morphotypes as, *okoppensis, bispinosum, bifidum* and *monospinosum* on the basis of caudal processes. Thirteen species were the first record in Japanese cattle. The average number of individuals per 1 ml of rumen fluid was 5.4×10^5, and that of species per head of host was 17.2.

INTRODUCTION

Rumen ciliate faunae would be different among the species of their hosts and/or among the hosts inhabiting separated areas [1, 2]. Surveys and comparisons of rumen ciliate faunae of various ruminants in different regions should provide informations about phylogenetic relationships among rumen ciliates, because it is suggested that the composition of rumen ciliates has peculiarly differentiated in relatively limited habitates since transfaunation has been assumed to occur only by direct contact between the hosts [3].

Various races of cattle including Japanese Black and Japanese Brown for beef, and Holstein-Friesian for milk have been kept in various localities in Japan [4, 5]. Holstein-Friesian which originated in the Netherlands and Germany were imported to Japan mainly through United States of America in the last part of the 19th century [5]. Since then, they have been kept as the most popular dairy cattle in Japan, especially in Hokkaido.

The rumen ciliate faunae of Japanese cattle in Honshu and Kyushu have been surveyed by Imai *et al.* [6, 7], but not of those in Hokkaido. The present paper deals with the species composition of ciliates obtained from the Holstein-Friesian cattle in Hokkaido and includes descriptions of two new species with four new formae.

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MATERIALS AND METHODS

Samples were collected from 71 Holstein-Friesian cattle bred in Okoppe, Hokkaido, Japan by means of rumen puncture from 1985 to 1988. They were immediately fixed and stained in methylgreen-formalin-saline (MFS) solution [3]. For close examination of nuclei and for type specimens, a part of the samples in which new species were recognized was stained with Mayer's hematoxylin and prepared as permanent slides. Identification was made followed by Ogimoto and Imai [3], Dogiel [8], Kofoid and MacLennan [9–11], and Imai [12]. Terminology of the morphology and orientation of ciliates for description of the new species conformed to our previous papers [1, 2, 3, 13]. The total ciliate number was calculated by means of Fuchs-Rosenthal haemocyte counter chamber. To obtain the average value for the ciliate density under normal distribution, it was computed from each value of ciliate number converted into logarithms. The generic composition is

shown as the percentage of each genus in about 300 individuals.

RESULTS

Entodinium okoppensis n. sp. (Figs. 1 and 2)

Description: Body rectangular to nearly square. Ectoplasm forming one to three caudal spines or lobes with various size at the posterior end of body. Anterior end of body flattened or concave. Anterior lip hardly visible when adoral cilia retracted. Vestibulum fairly large and funnel-shaped extending vertically but slightly bending leftward. Rectum short and extending vertically to

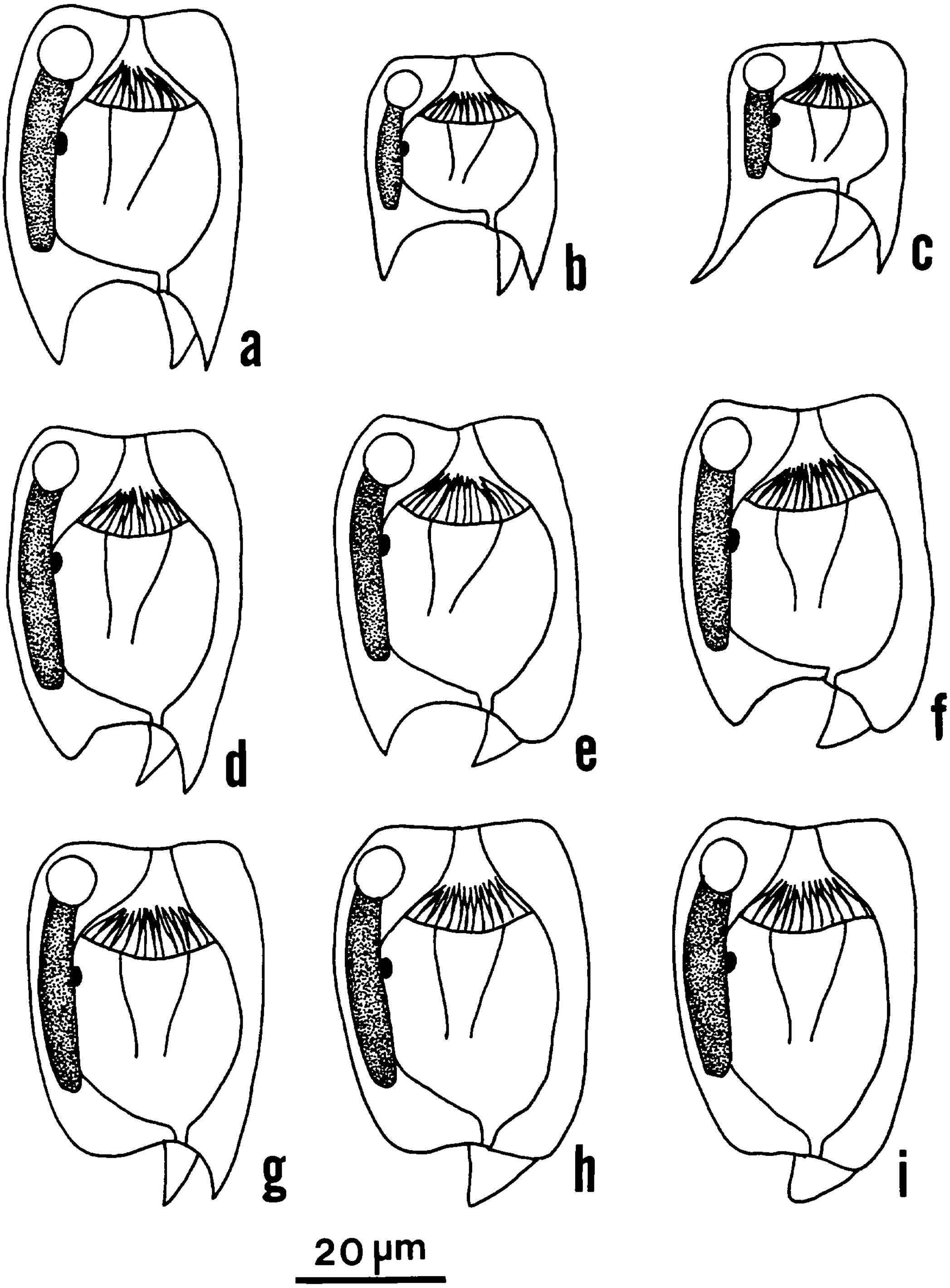

FIG. 1. *Entodinium okoppensis* n. sp. with its formae. a-d: forma *okoppensis*. e and f: forma *bispinosum*. g: forma *bifidum*. h and i: forma *monospinosum*.

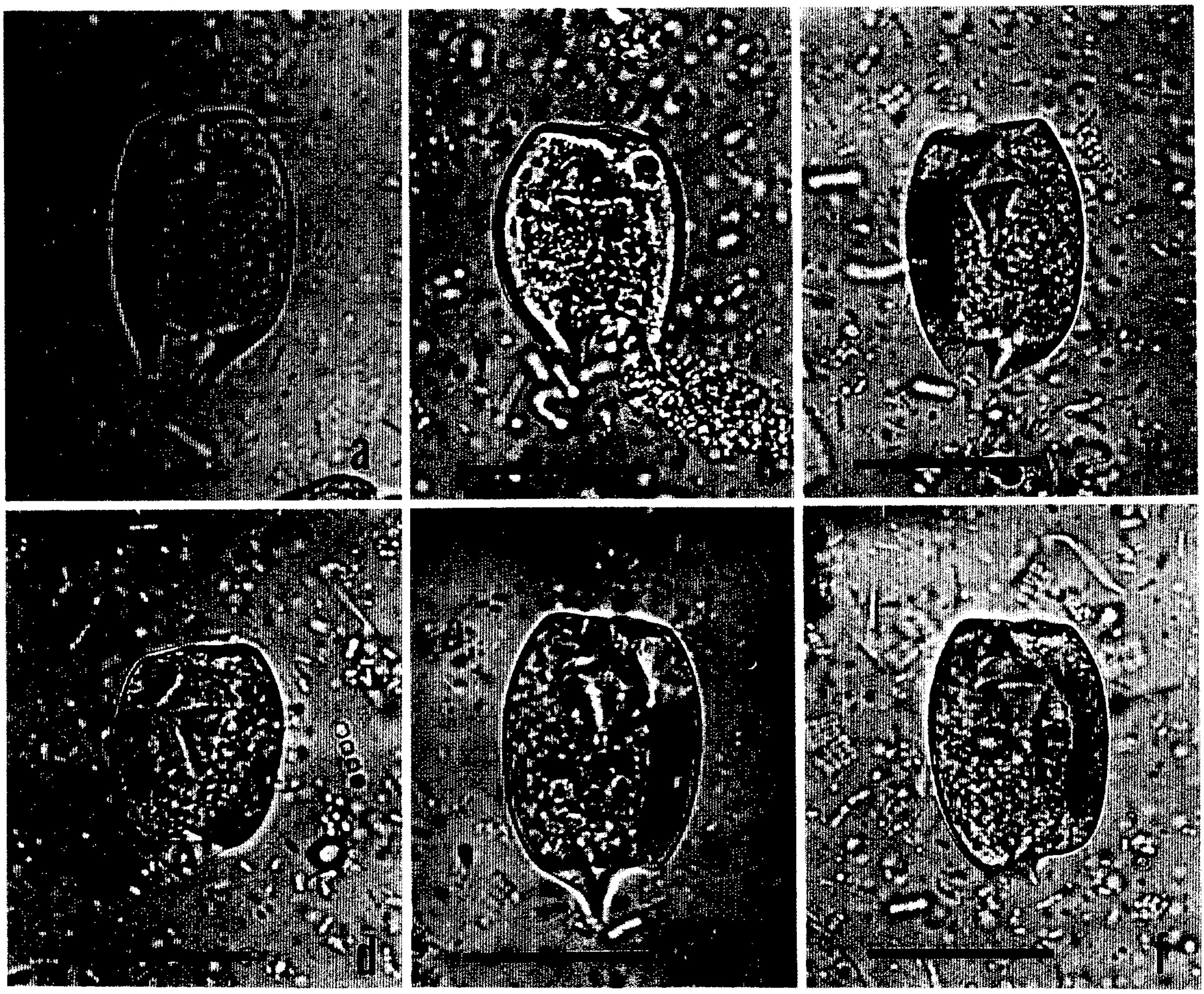

FIG. 2. Photomicrographs of *Entodinium okoppensis* n. sp. a and b: forma *okoppensis*. c and d: forma *bispinosum*. e: forma *bifidum*. f: forma *monospinosum*. All the specimens are fixed and stained with MFS solution. Bar in each figure indicates 30 μm.

cytoproct in the left side of median line. Macronucleus straight and slender rod shaped, four-fifths of body length, situated in the right periphery of body. Anterior end of macronucleus flattened, but posterior end rounded. An ovoidal micronucleus near left margin of middle of macronucleus. A contractile vacuole at just anterior and slightly upper to macronucleus.

Measurement: Body length 35.7±6.3 (24–55), caudal process 4.2±2.3 (1–10), width 26.2±3.0 (21–42) μm, length/width ratio 1.36±0.20 (0.9–1.7) (n=60).

Type specimens: Holotype, individual with retracted adoral cilia on microslide, No. 18901. Col. 5 MAR 1988, Ito, and 2 paratypes are deposited in the Department of Parasitology, Nippon Veterinary and Zootechnical College, Musashino, Tokyo, Japan.

Type host and locality: Holstein-Friesian cattle, *Bos taurus taurus*, in Hokkaido, Japan.

Habitat: Rumen.

Frequency: In 53.5% of the cattle surveyed.

Etymology: *Entodinium okoppensis* is named after the place this new species was found.

Four formae may be distinguished based on the number and shape of caudal processes.

Entodinium okoppensis forma *okoppensis* n. f.
(Figs. 1-a, b, c, d and 2-a, b)

Diagnosis: Three caudal processes; right one pointed or dull spine and sometimes bends outward, left-lower and left-upper ones pointed spines.

Frequency: In 53.5 % of the cattle surveyed.

Entodinium okoppensis forma *bispinosum* n. f.
(Figs. 1-e, f and 2-c, d)

Diagnosis: Three caudal processes; right one pointed or dull spine, left-upper one pointed spine, left-lower one blunt lobe.

Frequency: In 5.6 % of the cattle surveyed.

Entodinium okoppensis forma *bifidum* n. f.
(Figs. 1-g and 2-e)

Diagnosis: Two caudal spines in the same length at left side only.

Frequency: In 5.6 % of the cattle surveyed.

Entodinium okoppensis forma *monospinosum* n. f.
(Figs. 1-h, i and 2-f)

Diagnosis: One pointed or dull caudal spine at left-upper side.

Frequency: In 5.6 % of the cattle surveyed.

Remarks: *Entodinium okoppensis* closely resembles *Entodinium indicum* Kofoid et MacLennan, 1930 [9] and *E. bubalum* Imai, 1981 [14] in the shape of body and macronucleus, and the position of contractile vacuole. It is, however, distinguished from both *E. indicum* and *E. bubalum* by the position of the cytoproct and of caudal spines; that is, the largest caudal spine of both species is situated at the center of body, and the cytoproct lies at the center of body within the central spine. The body shape of *E. okoppensis okoppensis* also resembles *E. triacum* Buisson, 1923 [8, 15, 16], especially *E. triacum dextrum* Dogiel, 1927 [8]. Unfortunately, none of descriptions on the position of contractile vacuole which is one of the most important taxonomic criteria were made in *E. triacum* [8, 15, 16]. However, anterior end of *E. triacum* is more rounded than *E. okoppensis,* and the adoral lips are visible in *E. triacum* when the ciliate contracts the adoral cilia [8] but not visible in *E. okoppensis*. *Entodinium okoppensis bifidum* resembles *E. bifidum* Dogiel, 1927 [8] in the possession of two caudal spines at the posterior left end, but it is easily distinguished from *E. bifidum* by the difference in body shape and the location of contractile vacuole.

Taxonomical comment: The caudal process is one of the most prominent features under the microscope. However, it is clearly shown that the variation of caudal processes is continuous [3, 17, 18], thus these features are considered to be not suitable as the taxonomical character for species at present. In addition. the variation of caudal processes is recognized within one and the same host as also shown in *E. okoppensis* in the present examination, so that it also may not be able to be established as the character for subspecies. Therefore, the features of caudal processes seem to be the most adequate when they are used as the classification of formae which are not prescribed by the International Code of Zoological Nomenclature. There are some opinions that the establishment of formae is not necessary. However, the caudal processes seem to be related to the differentiation of rumen ciliates within the hosts, since a peculiar spinated form, such as *Epidinium ecaudatum capricornisi* has been found from only one host, Japanese serow [19], in spite of very wide distribution of other formae of this species, such as *E. ecaudatum ecaudatum* and *E. ecaudatum caudatum* [3]. Thus, the authors consider that it is significant to create formae in a species of rumen ciliates.

Ostracodinium munham n. sp.
(Figs. 3 and 4)

Description: Body ovoid. An apparent round lobe to the right of cytoproct at posterior end of body. Lobe with some variations (Fig. 3-b, c). Left posterior end of body rounded and sometimes projecting (Fig. 3-d). Large, slightly flattened operculum at top of body. Clear lips surrounding peristome and left ciliary zone when the ciliate contracted. Rectum clear and forming a short slit. Macronucleus rod-shaped tapering posteriorly with bend some degree along the left periphery of ectoplasm. An elliptical micronucleus at lower side of the middle of macronucleus. Three con-

tractile vacuoles in tandem in ectoplasm at lower-left side of macronucleus. Most anterior and posterior vacuoles at same level as anterior and posterior ends of macronucleus. Broad skeletal plate over most of upper part of body forming a weak arc and running obliquely to long axis from just posterior of operculum to the level of the posterior contractile vacuole. Left edge of the plate turning inward and extending one-fifth of the width of plate.

Measurement: Body length 78.7±7.5 (65–90), width 54.6±4.3 (50–65) μm, length/width ratio 1.43±0.08 (1.3–1.6)(n=20).

Type specimens: Holotype, individual with re-

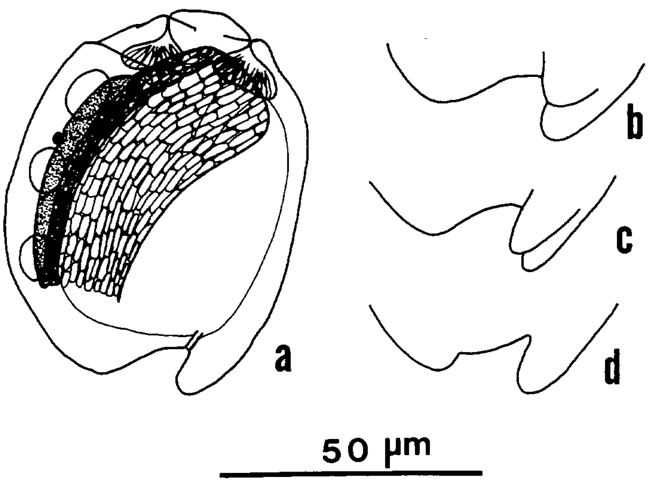

FIG. 3. *Ostracodinium munham* n. sp. a: Upper view of whole body. b-d: Variations in the caudal lobes.

tracted adoral cilia on microslide, No. 18902. Col. 18 JAN 1988, Ito, and 6 paratypes are deposited in the Department of Parasitology, Nippon Veterinary and Zootechnical College, Musashino, Tokyo, Japan.

Type host and locality: Holstein-Friesian cattle, *Bos taurus taurus*, in Hokkaido, Japan.

Habitat: Rumen.

Frequency: In 2.8% of the cattle surveyed.

Etymology: *Ostracodinium munham* is named after the possesion of lobe. Munha means a lobe in Aino.

Remarks: This new species closely resembles *Ostracodinium iwawoi* Imai, 1988 [13], in size and shape of body and of skeletal plate, but *O. iwawoi* differs from *O. munham* in its four contractile vacuoles and a skeletal plate turning one-third of its width. It also resembles *O. trivesiculatum* Kofoid et MacLennan, 1932 [10], *O. rugoloricatum* Kofoid et MacLennan, 1932 [10] and *O. mammosum* (Railliet, 1890) [8, 10], in having three contractile vacuoles. However, it differs from *O. trivesiculatum* in the folded skeletal plate and the presence of posterior lobe, from *O. rugoloricatum* in possessing no skeletal plate turning toward the middle of body, and from *O. mammosum* in shape of skeletal plate, and shape and number of posterior lobe of ectoplasm. In the point of possession of a right posterior lobe, the present species is also common with *O. obtusum* f.

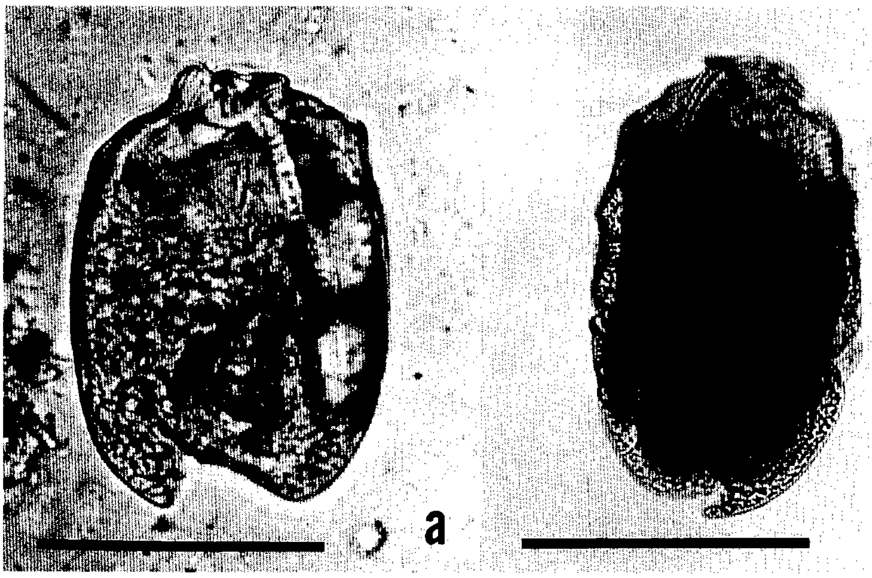

FIG. 4. Photomicrographs of *Ostracodinium munham* n. sp. a: Cell fixed and stained with MFS solution. b: Cell stained with Mayer's hematoxylin. Bar in each figure indicates 50 μm.

TABLE 1. Species composition and frequency of rumen ciliate protozoa found from the Holstein-Friesian cattle in Hokkaido

Family	Species	Frequency (%)
Isotrichidae	*Dasytricha*	
	ruminantium Schuberg	71.8
	Isotricha	
	prostoma Stein	66.2
	intestinalis Stein	33.8
	Oligoisotricha	
	bubali (Dogiel)	9.9
	Microcetus	
	lappus Orpin et Mathiesen*	1.4
Blepharocorythidae	*Charonina*	
	ventriculi (Jameson)	59.2
Ophryoscolecidae		
Entodiniinae	*Entodinium*	
	nanellum Dogiel	100.0
	simplex Dogiel	100.0
	parvum Buisson	95.8
	longinucleatum Dogiel	95.8
	caudatum	
	f. *caudatum* Stein	85.9
	f. *lobosospinosum* Dogiel	77.5
	rostratum Fiorentini	76.1
	exiguum Dogiel	60.6
	dilobum (Dogiel)	54.9
	okoppensis n. sp*	
	f. *okoppensis* n. f.	53.5
	f. *bispinosum* n. f.	5.6
	f. *bifidum* n. f.	5.6
	f. *monospinosum* n. f.	5.6
	bursa Stein	33.8
	ovinum Dogiel	19.7
	bimastus Dogiel	12.7
	minimum Schuberg	11.3
	chatterjeei Das-Gupta*	11.3
	bovis Wertheim*	5.6
	rectangulatum Kofoid et MacLennan*	4.2
	simulans Lubinsky*	2.8
	dubardi Buisson	1.4
	quadricuspis Dogiel*	1.4
Ophryoscolecidae		
Diplodiniinae	*Diplodinium*	
	anisacanthum Da Cunha	
	f. *anisacanthum* Da Cunha	50.7
	f. *monacanthum* Dogiel	19.7
	f. *anacanthum* Dogiel	16.9

	f. *diacanthum* Dogiel	7.0
	f. *triacanthum* Dogiel	7.0
	f. *tetracanthum* Dogiel	7.0
	f. *pentacanthum* Dogiel	5.6
	dentatum (Stein)	5.6
	minor (Dogiel)	4.2
	Eodinium	
	lobatum Kofoid et MacLennan	52.1
	posterovesiculatum (Dogiel)	46.5
	monolobosum (Hsiung)*	8.5
	rectangulatum Kofoid et MacLennan*	1.4
	Eudiplodinium	
	rostratum (Fiorentini)	84.5
	dilobum (Dogiel)	50.7
	maggii (Fiorentini)	42.3
	bovis (Dogiel)	19.7
	monolobum (Dogiel)	16.9
	Polyplastron	
	multivesiculatum (Dogiel et Fedorowa)	39.4
	Metadinium	
	affine Dogiel et Fedorowa	39.4
	medium Awerinzew et Mutafowa	7.0
	ypsilon (Dogiel)*	7.0
	Ostracodinium	
	mammosum (Railliet)	45.1
	gracile Dogiel	39.4
	trivesiculatum Kofoid et MacLennan*	21.1
	obtusum (Dogiel et Fedorowa)	19.7
	clipeolum Kofoid et MacLennan	5.6
	munham n. sp.*	2.8
	Enoploplastron	
	triloricatum (Dogiel)*	1.4
Ophryoscolecidae		
Ophryoscolecinae	*Epidinium*	
	ecaudatum (Fiorentini)	
	f. *caudatum* Fiorentini	25.4
	f. *ecaudatum* Fiorentini	18.3
	f. *cattanei* Fiorentini	7.0
	f. *bulbiferum* Dogiel	4.2
	f. *quadricaudatum* Sharp	2.8
	f. *hamatum* Schulze	1.4
	Ophryoscolex	
	purkynjei Stein	14.1
Total genera, species and formae		15 genera 50 species 19 formae

* First record in Japanese cattle.

TABLE 2. Percentage generic composition of the rumen ciliate protozoa in the Holstein-Friesian cattle in Hokkaido*

Genus	Mean	Range
Entodinium	82.7	43.6–98.6
Eudiplodinium	4.6	0 –17.7
Diplodinium	2.5	0 –24.7
Dasytricha	2.0	0 – 8.0
Epidinium	2.0	0 –26.7
Ostracodinium	1.7	0 – 6.7
Eodinium	1.5	0 – 6.7
Isotricha	1.2	0 – 9.3
Charonina	0.6	0 – 4.0
Metadinium	0.6	0 – 4.7
Oligoisotricha	0.2	0 – 4.7
Polyplastron	0.2	0 – 2.0
Microcetus	0.1	0 – 0.7
Enoploplastron	0.1	0 – 2.0
Ophryoscolex	0.1	0 – 2.3

* n=71.

monolobum Dogiel, 1927 [8], but it is easily discriminated from *O. munham* in different body size and number of contractile vacuoles.

Composition of rumen ciliates. Species and their frequency from 71 Holstein-Friesian examined are shown in Table 1. Fifty species with 19 formae under 15 genera were identified in all. Of them, 13 species were the first record in Japanese cattle. *Entodinium simplex* and *E. nanellum* occurred in all the hosts examined. Of the other ciliates, 6 species with 2 formae; *Entodinium parvum, E. longinucleatum, E. caudatum* f. *caudatum, E. caudatum* f. *lobosospinosum, E. rostratum, Eudiplodinium rostratum* and *Dasytricha ruminantium,* were predominant, and the frequencies of them were over 70% in the animals examined.

Table 2 shows the percentage compositions of genera of the ciliates in this examination. The percentage occupied by the genus *Entodinium* was the highest, the ratio of which was 82.7% on average. Though average ratio of the genus *Eudiplodinium* was next highest in number, *Epidinium* and *Diplodinium* occasionally showed higher ratio depending on the host individual.

The average number of species per head of host and the total ciliate number per one milliliter of rumen fluid are shown in Table. 3. The average number of ciliate species was 17.2, and the average ciliate density of 71 samples was 5.4×10^5/ml.

DISCUSSION

Rumen ciliate compositions of the cattle in Japan, mainly in Honshu were formerly surveyed on various races without distinction by Imai *et al.* [6, 7], and 42 species with 12 formae were identified. When the present results were compared to that by Imai *et al.* [6, 7,], 37 species were common in both areas, and the prominent species almost coincided with each other; included are several species of the genus *Entodinium, E. nanellum, E. parvum, E. simplex* and *E. longinucleatum.*

Thirteen species with 7 formae including 2 new species were the first record in Japanese cattle. Of those, *Entodinium bovis, E. rectangulatum, E. simulans, E. quadricuspis, Mocrocetus lappus, Metadinium ypsilon* and *Enoploplastron trilorica-tum* have been already detected from the various races of humpless cattle (*Bos taurus taurus*) in various areas [8, 20–22]. *Microcetus lappus* was first described from the cattle in Norway [22], and the present detection places the second report of this species.

Entodinium chatterjeei, Eodinium monolobosum, Eod. rectangulatum, and *Ostracodinium trivesiculatum* have been found mainly from the

TABLE 3. Average number of species appeared and average cilliate density in the Holstein-Friesian cattle in Hokkaido*

Number of species		Ciliate density ($\times10^4$/ml)	
Mean	Range	Mean	Range
17.2	5–30	53.7	14.5–168.2

* n=71.

domestic animals kept in tropical area [1, 2, 10, 13, 23, 24] but few from humpless cattle. There may be two possible reasons for this. One is that Holstein-Friesian cattle in Hokkaido might have an experience of contact with the cattle introduced from any tropical area, and the other is that these ciliate species are originally worldwide as well as *E. nanellum* and *E. simplex*. However, the former possibility seems to be low, because Holstein-Friesian cattle have been kept thoroughly in the temperate zones, and have had very few chances to contact with the tropical cattle. The high frequency of appearance of *E. okoppensis* was characteristic of the rumen ciliate fauna of the cattle in Hokkaido. The morphological features of the anterior end of body and macronucleus, and the position of contractile vacuole of *E. okoppensis* closely resemble those of *E. indicum* and *E. bubalum*. These two species have also been detected mainly from the animals inhabiting tropical areas [1, 2, 9, 13, 14], thus it seems to have a poor relationship to Holstein-Friesian. However, it will be interesting that *Campylodinium ovumrajae,* a closely related species to *Entodinium* and detected only from the forestomach of camels [8], has very similar morphological aspects on the anterior end of body, macronucleus and the position of contractile vacuole. Provided these species are assumed to reflect their phylogenetic relations in spite of their phylogenetically separated hosts, we can speculate that such morphotype is of primitive group and must have a wide distribution in various ruminants. This speculation would be supported by the fact that *E. triacum* with two formae which has been widely detected from the cattle in Europe [8, 25], Mexico [26] and China [23] also resembles *E. okoppensis,* although the description of *E. triacum* has been insufficient [8, 15, 16].

The average of the ciliate density and the average number of species per head of the host resembled those in the cattle in Honshu and Kyushu reported by Imai *et. al.* [6, 7]. The percentage composition of genera also almost coincided with the data from the Japanese cattle reported earlier by us [7]. It is known that the ciliate density and the composition of genera are strongly affected by the kinds and amounts of food taken by the host [27–29], and that when the host is fed with concentrates-rich ration, entodiniid ciliates rapidly grow, and the ratio of entodiniid ciliates and the total ciliate density become higher [29]. The similarity of ciliate percentage composition in various areas of Japan may be due to the similarity of feeding conditions. Frequent introduction of the Japanese dairy cattle from Hokkaido to Honshu may be another reason.

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